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Enhancing enzymatic hydrolysis of xylan by adding sodium lignosulfonate and long-chain fatty alcohols

Hongming Lou [a,b], Long Yuan [a], Xueqing Qiu [a,b,*], Kexian Qiu [a], Jinguo Fu [c], Yuxia Pang [a], Jinhao Huang [a]

[a] School of Chemistry and Chemical Engineering, South China University of Technology, Guangzhou, China
[b] State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou, China
[c] Guangdong Key Laboratory of Sugarcane Improvement & Biorefinery, Sugarcane Industry Research Institute, Guangzhou, China

HIGHLIGHTS

- SXSL and LFAs could enhance the enzymatic hydrolysis of xylan.
- SXSL showed synergism with LFAs on enhancing enzymatic hydrolysis of xylan.
- The cellulose and lignin seriously impede enzymatic hydrolysis of xylan.
- Cellulase could break the plant cell wall and make additives work better.

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ABSTRACT

Sodium lignosulfonate (SXSL) and long-chain fatty alcohols (LFAs) could enhance the enzymatic hydrolysis of xylan, and the compound of SXSL and LFAs have synergies on the enzymatic hydrolysis. SXSL shows a strong enhancement in buffer pH range from 4.0 to 6.0. The enhancement increased with the SXSL dosage and the xylanase loading. The cellulose and lignin in corncob substrate could not only adsorb xylanase nonproductively, but also seriously reduce the accessibility of xylanase on xylan to impede the enzymatic hydrolysis of xylan. Cellulase could break the plant cell wall structure of corncob and make additives work better. The xylose yield of corncob at 72 h increased from 59.4% to 73.7% by adding the compound of 5 g/L SXSL and 0.01% (v/v) *n*-decanol, which was higher than that without cellulase and additives by 30.7%. Meanwhile, the glucose yield at 72 h of corncob increased from 45.8% to 62.3%.

1. Introduction

Lignocellulosic biomass as an abundant and sustainable resource for the production of bioenergy and biomaterials has received extensive research and investment, with the growing depletion of fossil energy (Demirbas, 2008; Himmel et al., 2007; Ragauskas et al., 2006). Xylan is a major structural polysaccharide in plant cell walls, and it is the second most abundant polysaccharide in nature after cellulose, and can be converted to bio-fuels or xylitol and furfural through the sugar platform (Kallel et al., 2014; Li et al., 2014b). Xylan in many plants is heteropolysaccharide with homopolymeric backbone chains of β-1,4-linked xylose units (Hricovíniová, 2013). Xylan is the most abundant hemicelluloses which exists widely in agroforestry wastes, such as corncob, straw, bagasse and wood shavings (Saha, 2003). Corncob is one of the most abundant agricultural wastes containing about 30% xylan (Guo et al., 2013). Worldwide corn production has dramatically increased. For instance, about 5.92×10^8 and 9.67×10^8 t of the total global corn production had been reported in 2000 and 2013, respectively. Thus, corncob as agricultural waste has likewise increased significantly (Lin et al., 2015). Some corncobs are recycled and reused as bioproducts, whereas a large amount remains unused and burned in the fields, which may cause serious environmental problems (Li et al., 2011).

During the past decades, numerous researchers have devoted themselves to utilizing biomass by enzymatic hydrolysis (Van Dyk and Pletschke, 2012; Zhao et al., 2012) and making a deep exploration to improve enzymatic hydrolysis of lignocellulosic biomass (Lee et al., 2013; Li et al., 2014a; Mackenzie and Francis, 2012; Zhu and Pan, 2010). One promising approach to increase the enzymatic hydrolysis of cellulose is the supplementation of additives (Bornscheuer et al., 2014), such as by adding lignosulfonate (Lou et al., 2013, 2014c; Zhou et al., 2013),

* Corresponding author at: School of Chemistry and Chemical Engineering, South China University of Technology, Guangzhou, China. Tel.: +86 20 87114722; fax: +86 20 87114721.
E-mail address: xueqingqiu66@163.com (X. Qiu).

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long-chain fatty alcohols (Lou et al., 2014a,b), and non-ionic surfactants(Eriksson et al., 2002). This strategy has received much attention as it's effective and simple to operate (Börjesson et al., 2007; Li et al., 2012; Ouyang et al., 2010). Recently it was found that non-ionic surfactants (BSA, PEG 6000, and Tween 80) could improve the enzymatic hydrolysis efficiency of xylan in bamboo (Li et al., 2015a). However, there is a lack of information about the effect of anionic additive on the enzymatic hydrolysis of xylan. In addition, the effect mechanism of additives, cellulose and lignin on the enzymatic hydrolysis of xylan has not been clearly elucidated.

Corncob is large output and low price in China. The pretreated corncob could be used to produce xylose and glucose by enzymatic hydrolysis, as shown in Fig. 1. Those sugars could be converted into bioenergy, biomaterial and chemicals through chemical or biological processes. But lignin in corncob substrate was left as residue. Lignosulfonate can be prepared by sulfonation of the residual enzymatic hydrolysis lignin. This work intends to investigate the role of SXSL on the enhancement in the enzymatic hydrolysis efficiency of xylan, and implement the comprehensive utilization of lignocellulosic biomass, as shown in Fig. 1. At the same time, in order to reduce the negative impact of the foaming phenomenon of SXSL, the effect of antifoaming LFAs (Joshi et al., 2005) on the enzymatic hydrolysis of xylan were tested. The effects of additives on the non-productive adsorption of xylanase on cellulose and lignin were further investigated by UV–Vis Spectrophotometer and quartz crystal microbalance with dissipation monitoring (QCM-D), to reveal the underlying mechanism of enhancing the enzymatic hydrolysis of lignocelluloses. An efficient strategy was developed to improve the enzymatic hydrolysis of xylan in corncob by adding lignosulfonate and cellulase in combination.

2. Methods

2.1. Materials

Xylan (>90%) was purchased from Aladdin Industrial Corporation (Shanghai, China). Corncob was provided by Shengquan Corp. Ltd. (Jinan, Shandong province, China). Before enzymatic hydrolysis, the corncob was subjected to NaOH/Urea low temperature pretreatment (Li et al., 2010). It was processed successively by grinding and sieving (40–60 mesh), then immersed in 4 wt% NaOH/12 wt% urea aqueous solution at −16 °C for 40 min, and at last washed by deionized water until neutral pH. The content of cellulose, lignin and xylan in corncob was 38.3%, 23.4% and 13.4%, respectively. Crystalline cellulose (Avicel PH101) was purchased from Sigma-Aldrich (Shanghai, China), and 20 g of Avicel PH101 was milled in a planetary ball-milling system (QM-3SP4, Nanjing Nanda Instrument Plant, China), using 500 mL cups, 100 g spheres (15 mm in diameter) and 200 g spheres (10 mm in diameter). The ball-milling process was carried out for 4 h. Enzymatic hydrolysis lignin (Henan Tianguan Group Corp., Ltd, China) was prepared from the residue of simultaneous saccharification and fermentation of corn stover pretreated by steam explosion. The chemical compositions of enzymatic hydrolysis lignin were shown in Table 1. It was processed successively by filtration, washing, autoclaved sterilization, drying, grinding and sieving (40 mesh). The pure xylan was mixed with cellulose in the ratio of 2:3 and mixed with lignin in the ratio of 7:3, respectively, which were used as the simplified lignocellulosic substrates to research the impact of cellulose and lignin.

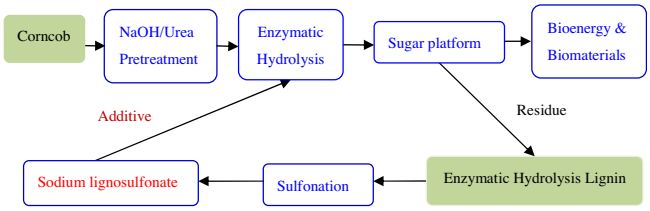

Fig. 1. The comprehensive utilization process of corncob.

Xylanase Accellerase® XY was provided by Genencor (Palo Alto, CA, USA), the protein concentration of xylanase is 42 mg/mL. Celluclast 1.5 L derived from *Trichoderma reesei* ATCC 26921 was bought from Sigma-Aldrich (Shanghai, China). The cellulase activity of Celluclast 1.5L was 117 FPU/mL.

n-Octanol (>99%), *n*-decanol (>99%) and *n*-dodecanol (>99%), marked as C8, C10 and C12 according to the number of carbon atoms in the alcohol, respectively, were purchased from Guangdong Guanghua Sci-Tech Co., Ltd. (China). Commercial sodium lignosulfonate (SXSL) from poplar sulfite pulping was produced by Shixian papermaking Corp. Ltd. (Yanbian, Jilin province, China). All chemicals were analytical grade and used as received. Milli-Q water was used for the preparation of all solutions.

2.2. Enzymatic hydrolysis

Enzymatic hydrolysis of xylan was conducted at 2% (w/v) in 50 mL of pH = 5.0, 50 mM acetate buffer on a shaker (DDHZ-300, Jiangsu Taicang equipment factory, China) at 50 °C and 200 rpm. Before adding xylanase, additives were first added to the mixtures of the substrate and acetate buffer, and then 0.1 g/L of tetracycline was added to retard bacterial growth. The xylanase loading was 1–15 mg protein/g xylan. Aliquots of 1 mL were taken at 72 h for xylose analysis after centrifuging at 10,000 rpm for 10 min.

2.3. Determination of xylose concentration

Xylose in enzymatic hydrolysate was measured by HPLC-ELSD. Xylose as standards was purchased from Sigma-Aldrich (Shanghai, China), the column was Prevail™ Carbohydrate ES (250 × 4.6, 5 μm), mobile phase was mixture of acetonitrile and water (70:30, v/v), flow rate was 1.0 mL/min, column temperature was 30 °C, injection volume was 10 μL, drift tube temperature was 80 °C, flow rate of carrier gas was 2.0 L/min. Xylose yield, defined as the percentage of xylan in the substrate enzymatically released to xylose, was used to represent the enzymatic hydrolysis efficiency of the substrate. Control experiments without additives (SXSL, LFAs) were also carried out for comparison. Each data point was the average of duplicates and the standard deviation was shown as error bar in the figures.

2.4. Preparation of the lignin film

Gold-coated QCM-D crystals sensors were supplied by Q-Sense AB (Sweden), with a thickness of 0.1 mm, a fundamental resonance frequency of 5 MHz, and sensitivity constant of 0.177 mg m^{-2} Hz^{-1}. Prior to spin-coating, the crystals sensors were immersed in a 5:1:1 mixture of water, hydrogen peroxide (30%) and ammonium hydroxide (25%) for 5 min at 75 °C and rinsed with Milli-Q water, then dried with nitrogen gas.

Table 1
The chemical compositions of enzymatic hydrolysis lignin.

Sample	Glucan (%)	Xylan (%)	Acid-insoluble lignin (%)	Acid-soluble lignin (%)	Ash (%)
Enzymatic hydrolysis lignin	5.3 ± 0.1	0.6 ± 0.1	72.0 ± 0.7	0.9 ± 0.1	19.8 ± 0.6